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State Lab Staff Assist Against AIDS in Africa

Patricia Somsel, Dr.P.H. and Patty Clark, MPH

Botswana is a country in Southern Africa about the size of Texas, located just north of South Africa, and surrounded by Namibia, Zambia and Zimbabwe. The land is predominantly flat and the Kalahari Desert is a prominent feature. Botswana has an impressive range of big game animals. But its most remarkable feature is its population, an open and friendly people, who currently face a devastating HIV burden. Today, life expectancy at birth has dropped below 40 years; without the impact of AIDS it would be over 70 years. Over 35 percent of pregnant women were HIV positive in 2002 and the rate in the population at large is estimated at over 40%.

In 1999, the Centers for Disease Control and Prevention (CDC) began an initiative for international collaboration on HIV/AIDS, the Global AIDS Program or GAP, to address laboratory infrastructure building and enhance services for HIV testing in 14 countries in Africa. The lead for the Botswana project, Frances Pouch Downes, Dr. P.H., State of Michigan Laboratory Administrator, has forged strong ties with laboratory staff and Ministry of Health officials in Botswana. A 2001 on-site assessment of laboratory infrastructure and capacity, led by Dr. Downes, provided recommendations for strengthening capacity and establishing a quality assurance program.

Botswana is aggressively expanding the implementation of rapid HIV testing in an effort to enable every citizen to respond to the challenge emblazoned on billboards and trucks: “Know Your Status.” Over 700 community sites will be supplied with test kits and high school graduate volunteers will be trained to administer the rapid tests at these sites. In order to meet the demand for a reliable stream of dependable kits, the Association of Public Health Laboratories (APHL) recruited members to travel to Botswana as consultants to BOTUSA, the CDC project in Botswana. Patty Clark, MDCH Viral Isolation/Viral Serology Unit Manager, along with Trish Somsel, Director of the Division of Infectious Diseases and Jane Getchell, Delaware State Laboratory Director, accepted the invitation and traveled to Botswana for two weeks in July, to work with staff of the National Blood Transfusion Center in a large-scale evaluation of available rapid test kits.

Jane Getchell, Patty Clark and Trish Somsel

With the high prevalence of HIV in Botswana, there was no shortage of positive samples. Rapid tests performed very well in this setting. However, it was the warmth and unflagging positive attitude of the people of Botswana that were the real surprises of the trip. Concern for the future of their country is never far from anyone’s thoughts, yet these laboratorians are optimistic and sunny. Their work hours are filled with far more laughter and chatter than is generally found in our laboratories.

Time spent working with laboratory colleagues in Botswana was punctuated by weekends observing big and small game in reserves in South Africa and in the northern Delta region of Botswana, where one of the world's largest concentration of elephants can be found at this time of year. Seeing lions, giraffe, and water buffalo from Land Rovers and hippopotamus and crocodile from watercraft were common weekend experiences, always led by the most remarkably knowledgeable local guides.

Despite over 20 hours spent in the air to reach Botswana, we are both eager to return to assist our colleagues there in their struggle with HIV. This pandemic will be played out first in the countries of Southern Africa. It is here that medical science is being called upon to interrupt its dreadful progression. High quality laboratory results are essential to direct this effort. As a recent APHL article explained, "a more fitting metaphor for the laboratory is the steering wheel of the car; since the laboratory data is the most objective measure of health status, it is the preeminent tool to guide the direction of virtually all HIV/AIDS interventions. What is the burden of infection in pregnant women? How long do high-risk individuals remain virus-free? Who is failing treatment and at risk of developing drug-resistant viral strains? The answers to these questions are dependent upon laboratory data."

Village of Odi

We can assist our colleagues in Africa by sharing our knowledge as clinical laboratorians, but most important is our sharing of their burden. APHL continues to seek laboratory professionals willing to become involved in HIV/AIDS overseas programs. While APHL generally works with public health laboratorians, professionals from clinical laboratories with strong interest and appropriate experience could be considered. The challenge to Botswana and other African nations is great and one that demands special skills and knowledge.

HIV-1 and HIV-2 Plus O Testing

R. Kenneth Terpstra, MS
Laboratory Manager/Technical Consultant
Kent County Health Department Regional Laboratory

In May 2004, the Kent County Health Department Regional Laboratory in Grand Rapids and the City of Detroit Health Department Laboratory began using a new version of the recombinant and synthetic peptide enzyme immunoassay (EIA) for the detection of antibody to Human Immunodeficiency Virus Types 1 and/or 2 in human serum and plasma as well as cadaveric specimens. Since 1986, two major HIV types, type-1 and type- 2, have been recognized. Sequence analysis of HIV-1 resulted in the classification of this virus into three groups: M (for major which includes subtypes A through J), O (for outliers) and N (for non-M, non-O). In addition to HIV-1 group M and HIV-2, this new generation of the assay allows for the detection of antibody to HIV-1 group O and is referred to as HIV-1, HIV-2 Plus O (Note: This is O, not Zero). Use of this new test will improve the sensitivity and specificity of antibody detection for both viruses and further aid in the diagnosis of HIV infection. Specimen requirements for the assay remain the same: 0.5 mL of serum or plasma. Both laboratories will continue to follow the CDC/APHL algorithm for confirmatory testing which includes repeating all initially-EIA-reactive specimens in duplicate and performing the HIV-1 Western Blot assay on samples where one or both of the repeat tests are reactive. For patients participating in incidence (STARHS) and baseline genotyping (VARS), 2.5 ml serum or plasma must be submitted. There will be no noticeable change to the laboratory reports since the reporting format for the assay has not changed. To avoid confusion we have opted not to include the "Plus O" in the name of the test. Questions related to any aspect of HIV serology can be addressed to Dr. Hema Kapoor (MDCH, 517-335-8099), Ms. Deborah Stephens (MDCH, 517-335-8098), Dr. Aloysius Hanson (City of Detroit Laboratory, 313-876-4223) or Kenneth Terpstra (Kent County Health Department Regional Laboratory, 616-336-3475).

Quirky bugs…
Inquilinus limosus ...A New Bacteria

Kathleen Russell MT (ASCP)
Reference Bacteriology Unit

Like all living things, bacteria recognize good neighborhoods or environments for their survival and growth. They grow, adapt and adjust as conditions change, or die out if their environment becomes too hostile. They make permanent changes (mutate) if they are able. Other bacteria may find the changed environment to their liking and move in and set up shop.

The lungs of people with cystic fibrosis (CF) offer an ecological niche (good neighborhood) for a variety of pathogens and also bacteria rarely seen in clinical samples from typical patients. CF is a genetic disorder that affects multiple organ systems, particularly the lungs and pancreas. Biochemical changes in the respiratory tract, accompanied by the production of abnormally thickened, viscous mucus, predisposes the lungs to chronic bacterial infections which are difficult or impossible to eradicate.

Burkholderia cepacia and similar related species (grouped together as *B. cepacia* complex) have been recognized since the 1980's as important agents in CF lung disease. *Staphylococcus aureus, Haemophilus influenzae* and mucoid strains of *Pseudomonas aeruginosa,* as well as other unusual bacteria not generally considered pathogens, are also found. These infections are difficult to treat. A high percentage of patients remain colonized with these bacteria for life, resulting in gradual deterioration of lung tissue and loss of pulmonary function.

B. cepacia complex strains can spread among CF patients in close proximity with one another. This has led to guidelines intended to reduce the spread of *B. cepacia* complex, such as discontinuation of summer camps for children with CF and segregation of colonized patients. Infection control measures make correct identification of isolates vital. Strict measures have reduced new infection rates. However, it is thought that CF patients can also acquire agents of infection from the environment. *B. cepacia* strains have been recovered from agricultural soil and it is known to cause soft rot in onions.

The Reference Bacteriology Unit at MDCH frequently receives isolates from CF patients. A recent submission, from the sputum of an otherwise medically stable 14 year-old cystic fibrosis patient, was particularly challenging and was referred to CDC. There it was identified as a possible *Inquilinus limosus*, a new genus and species first named in 2002 following a study of unusual respiratory bacteria from CF patients at the University of Michigan Medical School and Ghent University in Belgium. Its name reflects its interloper status: *Inquilinus* (In.qui=li.nus. L. masc. n. *inquilinus*, an inhabitant of a place that is not its own) *limosus* (li.mo=sus. L. masc. adj. *limosus*, full of slime, slimy).

The submitted isolate was slimy. It grew as a mucoid lactose non-fermenter on MacConkey and was also mucoid on chocolate and sheep blood agar at 35^0C and 25^0C. Gram stain morphology was a thick short ovoid gram-negative rod with bipolar staining. Biochemically, it was catalase and weakly oxidase positive. It slowly oxidized glucose, xylose, mannitol, lactose, sucrose and maltose. It was non-motile and indol, nitrate and nitrite negative. The Triple Sugar Iron (TSI) butt was negative for H_2S, but the lead acetate paper was positive for H_2S. It was lysine, ornithine and arginine negative. It was weakly urea positive at 4 days. It did not grow in 6% NaCl broth. Chromatography studies were inconclusive.

The altered environment of the CF lung is clearly "home" for unusual bacteria and for species not recognized as causing disease in humans. Accuracy in identification is a necessary part of infection control and treatment.

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PCR Improves Detection of *Bordetella pertussis*

James Rudrik, Ph.D.
Microbiology Section

In July 2003, MDCH began offering Polymerase Chain Reaction (PCR) testing for *Bordetella pertussis* from nasopharyngeal (NP) swabs sent to the laboratory in Regan-Lowe medium. The Regan-Lowe transport allows the laboratory to perform PCR and culture from the same sample. The results for 225 specimens tested between July 2003 and July 2004 are summarized in Table 1. PCR was positive for 15.1% (34/225) of specimens compared to 0.2% (5/225) for culture. In contrast, 56.4% of cultures were overgrown with other organisms that made detection of *B. pertussis* unlikely.

Table 1. Summary of results for specimens tested by PCR and culture

PCR Result				Culture Result		
Positive	**Negative**	**Indeterminate[a]**	**Inhibited[b]**	**Positive**	**Negative**	**Overgrown[c]**
34	179	3	9	5	93	127

[a] Duplicate samples tested, one result was positive and the other negative
[b] Amplification of internal control (beta-actin) inhibited by sample
[c] Culture overgrown with other organisms that prevented detection of *B. pertussis*

B. pertussis was detected in a total of 35 specimens; 30 by PCR only, 4 by culture and PCR, and one by culture only. Culture results for the nine inhibited samples, showed one positive, one negative and seven overgrown specimens. The culture results for the three indeterminate PCR results were one negative and two overgrown cultures. A comparison of culture and the positive PCR results is shown in Table 2. Culture failed to detect 30 of 35 (85.7%) total positives, while PCR missed only one positive (2.8%) in an inhibitory specimen.

Table 2. Culture results for PCR-positive specimens

	Culture Result		
PCR Positive	**Positive**	**Negative**	**Overgrown**
34	4	14	16

PCR offers a significant improvement in sensitivity compared to culture and offers the advantage of a short turnaround time compared to 21 days for culture. While PCR is clearly the new “gold standard” for detection of pertussis, culture is still necessary for some applications. Culture may be useful for PCR specimens that are inhibitory or indeterminate and is still needed for epidemiological investigations and susceptibility testing.

Appropriate specimen collection is important for maximum sensitivity for both culture and PCR. Specimen collection Unit 15, available from MDCH, contains an NP swab, Regan-Lowe medium, a test requisition, and directions for specimen collection and shipping. Validation studies at MDCH have shown a decrease in PCR sensitivity when NP swabs were submitted in Amies charcoal transport medium. Specimen collection kits may be obtained by contacting the laboratory support unit (phone 517-335-9867, fax 517-335-9039, e-mail DietzR@michigan.gov). The laboratory support unit is open 8:00 a.m. until 4:30 p.m. Monday through Friday.

FUN FUNGI.....
Penicillium marneffei

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

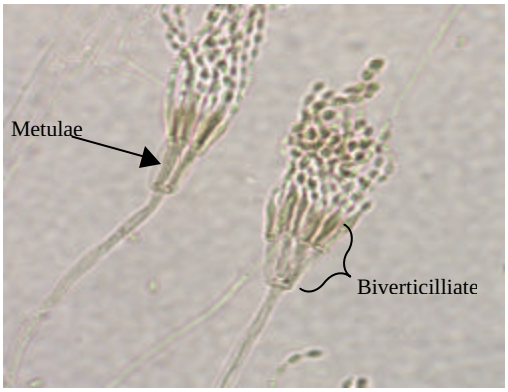
Penicillium marneffei, a human pathogenic fungus, is endemic to Southeast Asia (Thailand, Hong Kong, Vietnam, Indonesia, and the Guangxi Province of southern China). It is ecologically associated with bamboo rats. *P. marneffei* rarely causes infection in healthy individuals. It is the third most common opportunistic infection of HIV positive patients in northern Thailand. It has been associated with disseminated infection in immunocompromised travelers from France, Italy, Australia, the Netherlands, the United Kingdom and the United States who have been to endemic areas. The initial pulmonary infection is acquired by inhalation of conidia. The infection then disseminates to the reticuloendothelial system, and other deep organs. Some individuals develop acne-like papules on the face, trunk and extremities. Left untreated the disease is usually fatal.

P. marneffei, a thermally dimorphic fungus, grows as a mould at 25-30°C and as a yeast at 35-37°C on enriched media or *in vivo*. Growth at 25-30°C is rapid and appears powdery to velvety. Colonies are generally tan to yellow in color, with a brownish red reverse, but may also be greenish in color. The most identifiable feature is the deep red pigment that diffuses into the agar after three to seven days. Microscopically, *P. marneffei* resembles other *Penicillium* spp. The conidiophores of *P. marneffei* typically bear three to five metulae and the penicilli are biverticilliate. The conidia are ellipsoidal in shape.

There are a few other *Penicillium* spp. which develop a diffusible red pigment, most notably *P. purpurogenum*, but only *P. marneffei* converts to a yeast phase at 35-37°C. *P. marneffei* can be converted to the yeast phase by inoculating enriched media such as BHI with blood or cystine agar and incubating at 35-37°C. Conversion to the yeast phase can take up to 14 days. Colonies will be white to tan and yeast-like. Microscopically, the yeast cells will be round to oval with a central cross-wall giving them the appearance of arthroconidia. The yeast cells divide by fission not budding. The yeast cells are small, but may elongate and may become slightly curved.

***Penicillium marneffei* yeast phase**

***Penicillium marneffei* mould phase**



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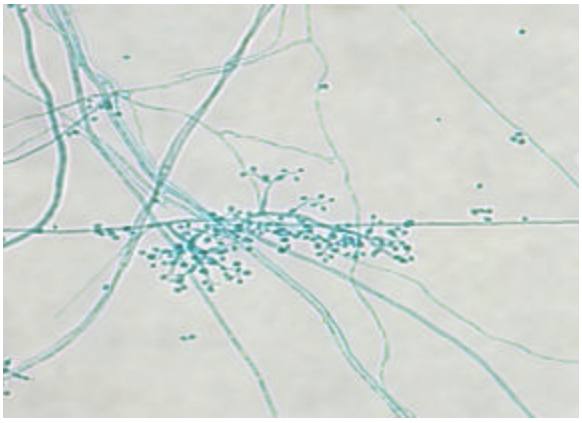
Last Issue's Picture Quiz Answer:

Nigrospora species

This picture is of the mould *Nigrospora*, a saprophyte typically isolated from decaying plant material and soil. This mould grows rapidly. The colony starts out white but develops brown to black pigment due to abundant sporulation. Some isolates can take up to three weeks of incubation before sporulating. The conidiophores are typically hyaline (may be slightly pigmented), short and inflated. The conidia are black, unicellular, ovoid to ellipsoidal and oblate (slightly horizontally flattened). The conidia also have a thin, equatorial germ slit, which is easier to visualize when the colony is young.

This Issues Picture Quiz: What Mould Is This?



This mould has moderately rapid growth. Colonies appear cottony to powdery and are white to pale pink or pale yellow in color. Microscopically, the conidiogenous cells have inflated bases and terminate in a rachis (zigzag filament). These cells are often grouped in masses. Conidia are hyaline and round to oval in shape.

Fall SCACM Meeting to be Held in Lansing

Martha Boehme, MT(ASCP)
Division of Infectious Diseases

Plan now to attend the fall Michigan meeting of the South Central Association for Microbiology (SCACM) on Tuesday, October 12 at the Holiday Inn South in Lansing. For 34 years, SCACM has provided continuing education to microbiologists working at the bench in hospital and other clinical laboratories. This year's theme, "Microbiology: The World is Our Backyard," emphasizes the need for all of us to stay informed. Emerging infectious disease issues and practical laboratory considerations will be included in formal presentations, while lunch will feature "buzz sessions" to allow informal sharing of ideas on several additional topics. Formal presentations include:

Agroterrorism Threats: The Microbiologist's Role on a Multi-Disciplinary Team (John Tilden, DVM, MPH, MI Dept. of Agriculture)

Antimicrobial Susceptibility Testing: Reporting Issues, Automated Systems, and Using "Expert" Rules (Janet Hindler, MT(ASCP),UCLA)

Ruts in the Road: How Three Different Laboratories are Coping with Implementation of the CDC Group B Beta Streptococcus Screening Guidelines (Therese Carson, Northern MI Hospital; Barbara Robinson-Dunn, Ph.D., William Beaumont Hospital; and Carol Young, U of MI Health System)

Down and Dirty-Antibiotic Use in the Barnyard: How does it Affect Us? (James Averill, DVM, MDCH)

Fun with Fungi; A Mycology Review (Sandra Arduin, MDCH)

There will also be a message board available for posting your position vacancies or other notices. Networking with other microbiologists is an important part of attending SCACM meetings!

Registration is handled directly through SCACM. Current individual SCACM members will receive program registration packets from them. MDCH has obtained CDC funding to provide scholarships for one person from each microbiology lab to attend this meeting. MDCH will send eligible laboratories a "coupon," good for one free admittance, to submit with the registration form.

Please contact Marty Boehme at 517-335-9654 or boehmem@michigan.gov if you would like to attend this meeting and have not received a registration packet from SCACM by mid-September.

Influenza Sentinel Surveillance

Hema Kapoor MD, Virology Section

Each year the Michigan Department of Community Health Bureaus of Laboratories and Epidemiology recruit physicians and laboratories to participate in seasonal influenza sentinel surveillance program. The goal of this program is to enroll physicians and laboratories from different areas of the state to monitor the subtype of influenza virus circulating throughout Michigan during the traditional influenza season- October until March. Monitoring disease activity and the viral types circulating assist in determining the severity of the on-going season, develop disease prevention recommendations, determine how well matched the current influenza vaccination formulation is to the circulating strains and predict the vaccine formula for the next influenza season.

Enrolled sentinel physicians are asked to submit clinical specimens and disease activity reports. Laboratories are asked to submit isolates during the influenza season for culture, identification, and sub typing. Physicians are provided with materials to collect and transport respiratory specimens from cases presenting with influenza like illness (ILI) three times per year; early (Oct), mid (Dec) and late (Feb) in influenza season. Sentinel physicians and practices are asked to collect two or three respiratory samples from patients with typical ILI early in the season; three more samples from the middle of the season when numbers of ILI cases are high; two or three more samples as ILI cases become less frequent toward the end of the season. Additionally, specimens from particularly severe or unusual cases are requested and tested at the MDCH virology laboratory.

Preliminary rapid results (with influenza type, A or B) are usually available 24 hours after the specimen arrives in our laboratory. In previous years, influenza direct fluorescent antigen tests (DFA) were performed on specimens received from sentinel physician sites and results were sent the day after specimens were received. The sensitivity of the DFA test is 40-70% depending on the prevalence of influenza in the community. Culture for respiratory viruses required seven days of observation and influenza viruses were recovered by day three at the earliest. This year we will begin utilizing new cell lines that require only five days observation. From initial testing, it was determined that 97.2% of influenza isolates appeared at 24 hours post inoculation of specimen with results in 100% agreement with the conventional tube method. Adenovirus and para influenza type 3 viruses were detected at 48 hours. This new cell system detected virus faster in all cultures tested.

Final influenza sub typing results will usually be available within 10 days. Additionally, it is recommended that sentinel physician offices receive lab reports electronically via fax instead of hard copy in the U.S. mail. This reduces delivery of results by 24 to 72 hours. To receive lab results by fax, contact Debbie Groh at 517-335-8059.

The Bureau of Laboratories is always looking for interested physician offices, health clinics, or other health care settings to participate in this program. If you are interested and would like more information, please contact Dr. Hema Kapoor, Virology Section Manager, at 517- 335-8099 or Patricia A. Clark Viral Serology /Viral Isolation Unit Manager, at 517-335-8102. Specific questions about the kits, or to request test kits, contact Bureau of Laboratories, 517-335-8067. For any other questions about the surveillance program contact Kyle Enger, 517-335-9449.

Now Available

The CD-ROM, *Mycology Review, Identification of Common Dermatophytes,* created by the Mycobacteriology/Mycology Unit, is now available through the National Laboratory Training Network's lending library (http://www.nltn.org) and on MI-TRAIN (http://mi.train.org) This CD-ROM covers 9 commonly seen dermatophytes and is useful

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

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